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ZINC METABOLISM AND THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE KIDNEYS OF WHITE RATS IN ALLOXAN DIABETES AND IN REPEATED ADMINISTRATION OF INSULIN

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ZINC METABOLISM AND THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE KIDNEYS OF WHITE RATS IN ALLOXAN DIABETES AND IN REPEATED ADMINISTRATION OF INSULIN

[Obmen Tsinka i Aktivnost' Shchelochnoy Fosfatazy v Pochkakh Belykh pri Alloksanovom Diabete i Povtornykh Vvedeniyakh Insulina]

by

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Experiments were performed on white rats; in animals with alloxan diabetes and following repeated insulin injections, the zinc distribution was studied histochemically, and the activity of alkaline phosphatase was studied by Gomori's method in the renal tubular cells. Zinc was distributed irregularly in the kidney: its highest concentration was found in the cells of the juxta-glomerular apparatus, in the margin of the cells in the main division of the nephron, in the ascending portion of Henle's loop, and in the tubules of the insertion portion. The highest alkaline phosphatase activity was found in the cells of the main division of the nephron. In alloxan diabetes, the zinc content and the alkaline phosphatase activity showed a sharp decrease, while in the case of repeated insulin injections these indices were observed to rise. A correlation was found between the alkaline phosphatase activity and zinc content in nephron tubules.

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Because of the important role of kidneys in maintaining homeostasis of the internal environment of the organism at an optimal level, the study of morphological and histochemical changes in the kidneys in alloxan diabetes and prolonged administration of insulin is of definite interest.

As we know [1-3], zinc plays a major role in cellular metabolism, since it is an activator of many enzyme systems and hormones. It was found [4] that zinc is found at its highest concentration in cells with endocrinal function and in certain cellular structures characterized by high functionality. The highest zinc content in the kidneys of man [5] and in animals [6] is noted in the region of the brush border of the cytoplasm of cells in the main segment of the nephron and in its juxta-glomerular apparatus.

Reabsorption of glucose in normal conditions occurs in the main (proximal) segment of the nephron, while glucose transport through the membrane of the tubules is carried out by means of alkaline phosphatase [7]. The highest amount of alkaline phosphatase is contained in cells of the main segment of the nephron [8]. The brush border showed the highest activity of this enzyme [9].

Materials and Methods

The study was conducted on 40 white male rats weighing from 100 to 150 grams. The animals were distributed in four groups: the first group was the control group; rats in the second group were given a single subcutaneous administration of 250 mg/kg alloxan; the animals in the third

group were injected daily, morning and evening, with 0.5 units of insulin for 10 days; rats in the fourth group -- after a single inhalation of alloxan at a dose of 250 mg/kg -- were administered twice daily 0.5 units of insulin each.

The fasting blood sugar level was determined during the experiment for all animals using the Hagedorn-Jensen method. After 10 days, all animals were sacrificed and the kidneys were removed for examination.

The zinc concentration and distribution was studied histochemically by the silver sulfide method [10]. Essentially, this method consists in tissue zinc being converted into zinc sulfate -- an active catalyst -- after fixation of the material in 70° alcohol saturated with hydrogen sulfide. After subsequent treatment of the material with a reagent consisting of solutions of hyposulfite, gum arabic (or cherry gum), and silver nitrate, the latter compound is reduced in regions with highest zinc concentration. The accumulation of zinc in the corresponding structures is estimated from the amount of brown granules of precipitating silver and by color intensity.

The activity of alkaline phosphatase was determined by a modified Gomori method [11]. The alkaline phosphatase activity was estimated by the intensity of the black color associated with the precipitation of cobalt sulfite.

Results and Their Discussion

As shown by the studies, zinc was unevenly distributed in the control animal group kidneys. Zinc was at a low concentration in the cells of the medullary tubules and in preparations treated with silver sulfide

the medulla appeared faintly yellowish (Figs. 1 and 2). The zinc concentration was higher in the convoluted tubules of the proximal and distal segments of the nephron and also in the cells of the descending portion of Henle's loop, and these segments were colored more intensely. Especially typically is the zinc distribution in the cytoplasm of the cells in the main nephron segment, where its highest concentration was observed in the apical zone of the cells and in the region of the brush border. An increased zinc concentration was noted also in the cytoplasm of cells in the juxta-glomerular segment of the afferent arteriole.

When the kidney preparations of experimental animals were studied, treated to reveal alkaline phosphatase, this compound was found mainly in the cytoplasm of the main segment of the nephron. The activity of alkaline phosphatase was negligible in the medullary tubules and also in the Henle cells and in the distal tubules of the nephron.

The fasting blood sugar level in second-group rats receiving 250 mg/kg alloxan averaged 450-700 mg percent on the third day of the experiment.

When preparations of kidneys treated by the silver sulfide method were examined, no increased zinc concentration was found. All nephron segments, and also the collecting tubules of the medulla showed no reaction to zinc and appeared just as light as the medulla in the kidneys of the control animals. In preparations treated by the Gomori method for alkaline phosphatase, the activity of the latter proved to be significantly reduced in all nephron segments.

A sharp rise in zinc concentration was noted in kidneys of animals in the third group -- receiving 0.5 units insulin each twice a day for

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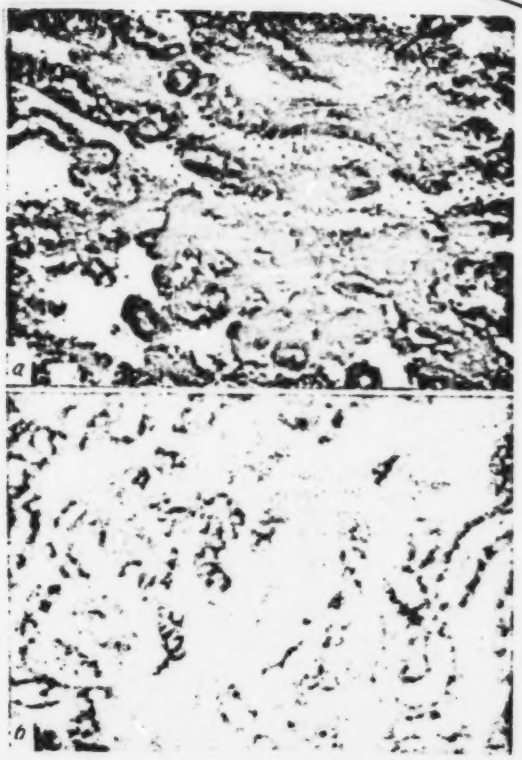

Fig. 1: Microphotograph. Activity of alkaline phosphatase in the tubule cells of white rat kidneys
a -- after repeated administrations of insulin
b -- in alloxan diabetes. Treatment by the Gomori method. Objective 20 X, ocular 10 X.

10 days -- in the tubule cells of the main segment of the nephron, the cells of the ascending Henle's loops, and the cells of the insertion segment. No zinc accumulation was found in the juxta-glomerular segments of the nephron. An especially high zinc concentration was determined in the cytoplasm of the cells in the main segment of the nephron. Compared with the control, the zinc concentration band in the apical zone of the cells in the main segment of the nephron was several times wider and darker in color.

Interestingly, in kidney preparations treated for alkaline phosphatase, the latter revealed its highest activity in the cytoplasm of the cells of the main segment of the nephron. The activity of the enzyme was negligible in other segments.

Results of studying the kidneys of animals in the fourth group, which received 0.5 units insulin each daily after the administration of 250 mg/kg of alloxan, differed little from the control both as to the concentration and distribution of zinc, as well as to the activity of alkaline phosphatase.

Conclusions

1. Zinc is unevenly distributed in the kidney. The highest zinc concentration was determined in the cells of the juxta-glomerular complex, the cells of the main segment of the nephron, the ascending portion of Henle's loop, and in the cells of the insertion segment. Zinc was found almost not at all in the collecting and papillary tubules and in the cells of the descending portion of Henle's loop.

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Fig. 2: Microphotograph. Reaction to zinc in the tubule cells of kidneys of a white rat
a -- for repeated insulin administrations
b -- in alloxan diabetes. Treatment by the silver sulfide method and hematoxylin.
Objective 20 X, ocular 10 X

The highest activity of alkaline phosphatase was found in the cells of the main segment of the nephron. The activity of the enzyme was negligible in the remaining cells.

2. In alloxan diabetes the zinc concentration in the main and the insertion segments of the nephron, and also in the ascending portion

of Henle's loop was reduced, and the latter region did not differ in zinc concentration from the collecting and papillary tubules. The activity of alkaline phosphatase in alloxan diabetes was reduced in the cells of the main segment of the nephron.

3. After prolonged administration of insulin, the zinc concentration in the main segments, the ascending portions of Henle's loop, and the insertion segments of the nephron rose sharply. An especially high zinc concentration was noted in the apical zone of the cells in the main segment of the nephron and in the region of the brush border. The activity of alkaline phosphatase in the kidneys rose sharply, concentrating mainly in the cells of the main segments of the nephron.

4. A parallelism was noted between zinc accumulation in the main segments of the nephron and the rise in alkaline phosphatase activity.

5. These data indicate a reduction in the reabsorptive function of the nephron tubules in alloxan diabetes and a rise in this function under the effect of insulin.

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